**Renewal Progress Report for CDFA Agreement Number 14-0486-SA**

# EXPANDING THE RANGE OF GRAPE ROOTSTOCK AND SCION GENOTYPES THAT CAN BE GENETICALLY MODIFIED FOR USE IN RESEARCH AND PRODUCT DEVELOPMENT

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**Time period covered by the report**: January 16, 2015 – February 16, 2016

## Introduction:

This proposal is aimed at applying the progress that has been made in grape cell biology and transformation technology of rootstock genotypes 1103P and 101-14 to additional grape rootstock genotypes in order to expand the range of genotypes amenable to transformation. This research will apply the pre-existing expertise and technical know-how developed for rootstocks 1103P and 101-14 at UC Davis's Plant Transformation Facility to additional rootstock germplasms important for the California wine industry. For this proposal, we are testing seven additional rootstocks for their amenability to transformation including; Richter 110 (clone 1), 3309C (clone 05), Freedom (clone 1), Harmony, MGT 420A (clone 4), 140Ru (clone 1) and Salt Creek (clone 8). In 2016 we will add rootstock genotype GRN-1 01.1 from Andy Walker's program. This work will expand the range of rootstocks that can be effectively transformed, allowing rootstock-mediated disease resistance technology to be employed across the major wine growing regions in California. Although a rootstock-mediated resistance strategy is the preferred mechanism for achieving resistance to Pierce's Disease in grape, investing in the development of transformation technology for scions will serve an important fallback position should rootstock-mediated resistance fail to confer adequate levels of resistance to the scion and direct transformation of scion varieties be required. Therefore, in addition to testing the utility of our tissue culture and transformation protocols on six additional grape rootstocks, we will also screen six important California scion genotypes for their amenability to transformation including; Cabernet Sauvignon (clone 07), Chardonnay (clone 04), Pinot Noir (2A), Merlot (clone 03), Zinfandel (clone 01A), and French Colombard (clone 02). Although it is unlikely that all seven rootstock and six scion genotypes will be amenable to transformation using our established protocols, we believe that a significant number will respond positively. The results of this work will allow for the establishment of grape tissue culture and transformation technologies that can be utilized by the PD/GWSS Research Community. It will also establish a germplasm bank of cell suspension cultures and a repository of somatic embryos for rootstock and scion genotypes used in California, which can be made available to the research community. To date, we have made significant progress in establishing somatic embryos, in vitro shoot cultures and bulk meristem cultures, cell suspension cultures and stored embryo germplasm bank for many of the targeted genotypes. We have now demonstrated that in addition to rootstocks 101-14 and 1103, that Richter can be included in the list of rootstock genotypes that we can successfully transform. Next year we will focus on establishing somatic embryos, cell suspensions, stored somatic embryos and bulk meristems from the remaining genotypes, while continuing to evaluate the transformation efficiency of this material with the final goal of adding 3309c 140 Ru, MGT 420A, Freedom, Harmony, and Salt Creek to our transformation portfolio.

**OBJECTIVES**

**1. Develop embryogenic cultures from anthers of seven rootstock genotypes and six scion genotypes for use in establishing embryogenic suspension cultures.**

**2. Develop embryogenic suspension cultures for seven rootstock genotypes and six scion genotypes, which will provide a continuous supply of somatic embryos for use in transformation experiments.**

**3. Establish a germplasm bank of somatic embryos for seven rootstock genotypes and six scion genotypes by plating aliquots of the cell suspension cultures on high osmotic medium.**

**4. Test transformation efficiencies of seven rootstock genotypes and six scion genotypes using our established somatic embryo transformation protocols.**

**5. Test direct cell suspension transformation technology on seven rootstock genotypes and six scion genotypes.**

**6. Establish in vitro shoot cultures for seven rootstock genotypes and six scion genotypes using indexed material from Foundation Plant Services (FPS) or field material from FPS and establish bulk meristem cultures for all 13 genotypes for use in transformation.**

**7. Test Mezzetti et al., 2002, bulk meristem transformation methodology for seven rootstock genotypes and six scion genotypes as an alternate to somatic embryo transformation.**

**Description of activities conducted to accomplish each objective and a summary of accomplishments and results for each objective.**

**Objective 1. Develop embryogenic cultures from anthers of seven rootstock genotypes and six scion genotypes for use in establishing embryogenic suspension cultures.**

**Progress:**

We collected anthers of rootstock genotypes 3309C (clone 05), Freedom (clone 01), Richter 110 (clone 01), MGT 420A (clone 04), 140Ru (clone 01), Salt Creek (clone 08) 11-03P, 101-14, and scion genotypes Cabernet Sauvignon (clone 07), Chardonnay (clone 04), Pinot Noir (clone 2A), Zinfandel (clone 01A), and Colombard (clone 04) and plated them on four different embryogenic callus inducing media. The media included; Nitsch and Nitsch minimal organics medium (1969) supplemented with 60 g/l sucrose, 1.0 mg/l 2,4-dichlorophenoxyacetic acid (2,4-D) and 2.0 mg/l benzylaminopurine (BAP) (PIV), MS minimal organics medium supplemented with 20 g/l sucrose 1.0 mg/l 2,4-D and 0.2 mg/l BAP (MSE), MS minimal organics medium supplemented with 30 g/l sucrose 1.0 mg/l 2,4-D and 1.0 mg/l BAP (MS1) or one half strength MS minimal organics medium supplemented with 15 g/l sucrose 1.0 mg/l NOA and 0.2 mg/l BAP (NB). Anthers were collected during two separate weeks in the spring of 2015. The number of anther clusters plated for each genotype is given in **Table 1**, and the number of putative embryogenic calli or embryos developing to date is provided in **Table 2**. Some line produced callus, but this callus failed to generate somatic embryos. However, we have successfully established stable somatic embryo cultures for rootstock genotypes Freedom, Richter 110R, MGT 420A, 140Ru 01, 1103P and 101-14 and scion genotypes; Cabernet sauvignon, Chardonnay, Colombard and Pinot Noir. In the spring of 2016 we will concentrate on establishing embryogenic cultures of rootstock genotype for which we were unsuccessful

in establishing somatic embryos in 2015 which includes; 3309C, GRN-1 01.1, Harmony and Salt Creek as well as scion genotypes Merlot, Cabernet sauvignon (07) and Zinfandel.

**Table 1.** Summary of the number of anther clusters plated onto four different media formulations

| Grape Anther Cultures | MSI | MSE | PIV | NB |
|---|---|---|---|---|
| | | | | |
| Zinfandel 01A R5V12 | 196 | 49 | 147 | 196 |
| Colombard 04 R1V13 | 123 | 49 | 172 | 123 |
| Pinot Noir 02A R6V17 | 96 | 49 | 196 | 147 |
| MGT 420A R2V3 | 196 | 98 | 147 | 196 |
| 140Ru 01 R2V7 | 196 | 98 | 245 | 196 |
| Freedom 01 R4V6 | 147 | 49 | 294 | 245 |
| 3309C 05 R1V3 | 196 | 0 | 196 | 196 |
| CAB 07 R2V12 | 147 | 147 | 98 | 196 |
| Salt Creek 08 R2V3 | 147 | 0 | 196 | 147 |
| TS 02A C1V21 | 98 | 49 | 98 | 98 |
| 110R R3V3 | 49 | 0 | 49 | 49 |
| 1103 A6V2 | 49 | 49 | 49 | 49 |
| 101-14 A5V5 | 49 | 0 | 49 | 49 |
| Total | 45 | 17 | 43 | 41 |

**Table 2.** Number (percentage) of anther clusters forming putative embryogenic callus or embryos

| Genotype | PIV | MSE | MS1 | NB |
|---|---|---|---|---|
| | | | | |
| 3309C (05)* | 1/196 (0.5) | 0/196 (0) | 0/196 (0) | 0/196 (0) |
| Freedom (01) | 1/294 (0.3) | 0/49 (0) | 0/147 (0) | 0/245 (0) |
| Richter 110 (01) | 0/49 (0) | | 0/49 (0) | 2/49 (4) |
| MGT 420A (04) | 1/147 (0.7) | 1/98 (1.0) | 5/196 (2.5) | 1/196 (0.5) |
| 140Ru (01) | 0/49 (0) | | 0/49 (0) | 0/49 (0) |
| Salt Creek (08)* | 5/196 (2.5) | | 4/147 (2.7) | 1/147 (0.7) |
| 11-03 | 0/49 (0) | 1/49 (2.0) | | 8/49 (16) |
| 101-14 | 0/49 (0) | | 0/49 (0) | 0/49 (0) |
| Cabernet Sauvignon (07) | 1/98 (1.0) | 4/147 (2.7) | 1/147 (0.7) | 1/196 (0.5) |
| Cabernet Sauvignon (08)* | 5/539 (0.9) | | | |
| Pinot Noir (2A) | 4/196 (2.0) | 0/49 (0) | 0/96 (0) | 6/147 (4.0) |
| Zinfandel (01A)* | 2/147 (1.7) | 0/49 (0) | 11/196 (5.6) | 0/196 (0) |
| Colombard (04) | 7/172 (4.1) | 0/49 (0) | 16/123 (13.0) | 2/123 (1.6) |

***To date callus generated on these genotypes has not transitioned into embryogenic callus**

**Objective 2. Develop embryogenic suspension cultures for seven rootstock genotypes and six scion genotypes, which will provide a continuous supply of somatic embryos for use in transformation experiments.**

**Progress:**

To date, we have established suspension cultures for rootstock genotypes Richter 110, 1103, 101-14, MGT 420A, 140Ru, and scion genotypes Cabernet sauvignon (clone 8), Chardonnay and Colombard. We are still in the process of bulking callus cultures for rootstock genotype 3309C, Freedom, and scion genotype, Pinot Noir. Once adequate amounts of embryogenic tissue have developed, we will initiate suspension cultures for these genotypes.

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**Objective 3. Establish a germplasm bank of somatic embryos for seven rootstock genotypes and six scion genotypes by plating aliquots of the cell suspension cultures on high osmotic medium.**

We have established a germplasm bank of somatic embryos on agar solidified Woody Plant Media (WPM) supplemented with 20 g/liter sucrose, 1g/liter casein hydrolysate, 500 mg/liter activated charcoal, 0.5 mg/liter BAP, 0.1 mg/liter NAA, 5% sorbitol and 14 g/l phytoagar (BN-sorb) for rootstock genotypes Richter 110, 1103, 101-14, MGT 420A, 140Ru and scion genotypes Cabernet sauvignon, Chardonnay and Colombard (**Figure 1**). These cultures will provide a reliable source of embryos for use in transformation studies. Once rapidly dividing embryogenic suspension cultures are initiated, we will establish a germplasm bank of stored somatic embryos for rootstock genotypes 3309C, Freedom and scion genotypes Pinot Noir (clone 2A).

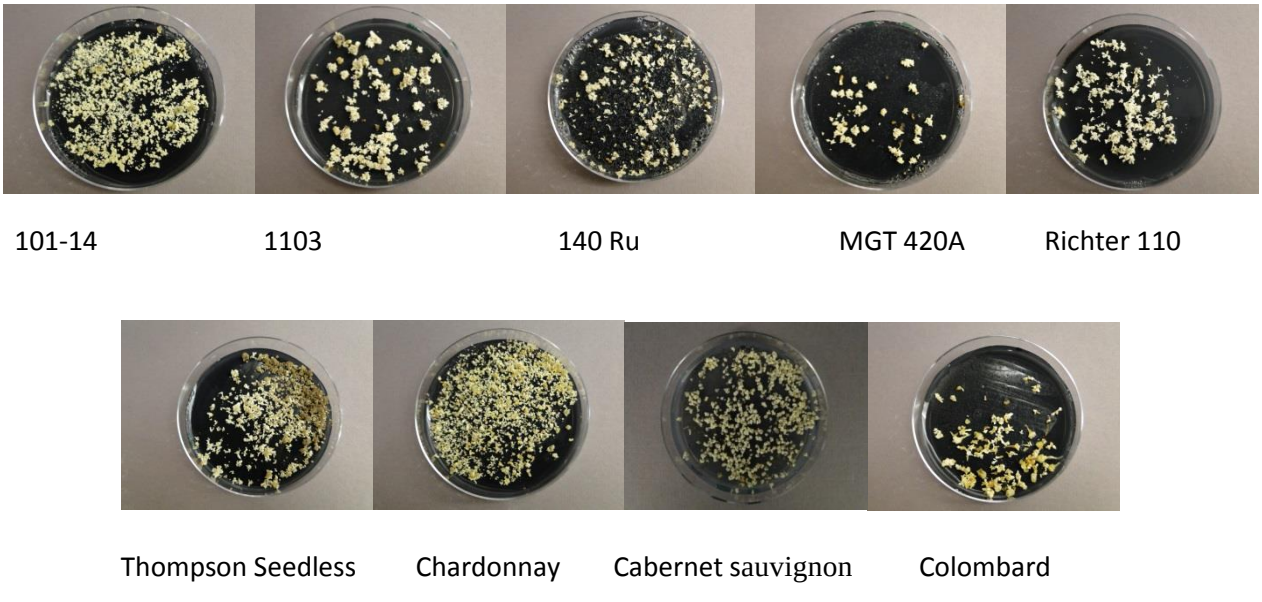


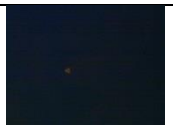

**Figure 1.** Germplasm bank of embryos established from grape suspension cultures plated on sorbitol containing medium

**Objective 4. Test transformation efficiencies of seven rootstock genotypes and six scion genotypes using our established somatic embryo transformation protocols.**

Transformation experiments have been initiated using known amounts of somatic embryos as determined by fresh weight for rootstock genotypes Richter 110, 1103, 101-14, MGT 420A, 140Ru and scion genotypes Chardonnay and Colombard using a construct containing the DsRed florescent scorable marker gene which will allow us to monitor the progress of transformation in real time without sacrificing

any tissue (**Table 3**). Thompson Seedless is being included as a positive control. DsRed expression is being evaluated at 1, 2, 3 and 4 months post inoculation. Once germplasm banks of somatic embryos are established, we will begin testing our transformation system on somatic embryos of rootstock genotypes 3309C, and Freedom, and scion genotypes Cabernet Sauvignon and Pinot Noir.

**Table 3.** Transformation experiments to assess the amenability of transformation of stored grape embryos for a range of rootstock and scion genotypes using the scorable fluorescent marker gene DsRed.

| Genotype | Date | Experiment # | Tissue Weight | Percentage of DsRed embryos | Photos of DsRed Expression | Regeneration of transgenic plants |
|---|---|---|---|---|---|---|
| | | | | | | |
| **TS-14** | 6/26/2015 | 159050 | 0.53 | 10% |  | Yes |
| | 7/24/2015 | 159070 | 0.52 | 20% | | |
| | 8/26/2015 | 159096 | 0.92 | 20% | | |
| | | | | | | |
| **Chardonnay** | 6/26/2015 | 159048 | 2.72 | 0% |  | No |
| | 7/10/2015 | 159064 | 1.12 | 0% | | |
| | 7/17/2015 | 159068 | 1.12 | <1% | | |
| | 7/24/2015 | 159071 | 0.57 | 0% | | |
| | | | | | | |
| | | | | |  | |
| **Richter** | 7/10/2015 | 159065 | 1.65 | 25% | | yes |
| | 7/17/2015 | 159069 | 1.83 | 25% | | |
| | 7/24/2015 | 159072 | 0.42 | 30% | | |
| | 8/26/2015 | 159095 | 0.89 | 5% | | |
| | | | | | | |
| **1103** | 7/24/2015 | 159073 | 1.11 | 10% |  | Yes |
| | 8/26/2015 | 159093 | 1.09 | 10% | | |
| | | | | | | |
| **Colombard** | 12/16/2015 | 159150 | 1.96 | | | In progress |
| | 1/15/2016 | 169007 | 0.55 | | | |
| | | | | | | |
| **140 Ru** | 12/16/2015 | 159151 | 1.49 | | | In progress |
| | 1/15/2016 | 169008 | 0.92 | | | |
| | | | | | | |
| **MGT 40A** | 12/16/2015 | 159152 | 0.53 | | | In progress |
| | 1/15/2016 | 169009 | 0.21 | | | |
| | | | | | | |
| **101-14** | 7/24/2015 | 159074 | 0.86 | 10% |  | Yes |
| | 8/26/2015 | 159094 | 0.97 | 10% | | |

**Objective 5. Test direct cell suspension transformation technology on seven rootstock genotypes and six scion genotypes.**

We are trying to leverage the progress we have made in developing high quality cell suspensions that have the ability to rapidly regenerate whole plants when plated onto agar-solidified medium by directly transforming our grape cell suspension cultures with the scorable marker gene DsRed. Ten mls of a grape cell suspension grown in liquid Pic/MT medium, containing pre-embryogenic masses or small globular embryos are collected in a 15 ml conical centrifuge tube and pelleted by centrifugation at 1000 x G for 3 minutes. The cells are subjected to heat shock by placing the conical tube in a 45 degree water bath for 5 minutes. After heat shock the supernatant is removed and replaced with 5 ml liquid BN medium containing 200 uM acetosyringone and the *Agrobacterium* strain and appropriate vector at an $OD_{600}$ of 01.-0.2. The suspension is centrifuged at 1000 x G for 5 minutes and allowed to incubate for 25 minutes at room temperature. After 25 minutes all but 0.5 ml of the supernatant is removed. The grape and *Agrobacterium* cells are then re-suspended and transferred to sterile Whatman filter paper in an empty 100 x 20 mm petri dish. Any excess fluid was carefully bloated up with a second sterile filter paper. The plates are co-cultured in the dark for 2-3 days at 23 degrees and then transferred to selection medium consisting of WPM supplemented with 20 g/l sucrose, 1g/l casein, 1M MES, 500 mg/l activated charcoal, 0.5 mg/l BAP, 0.1 mg/l NAA, 400 mg/l carbenicillin, 150 mg/l timentin, 200 mg/l kanamycin, 50 g/ sorbitol and 14 g/l agar. The filter paper is transferred to fresh medium every 2 weeks. Within eight weeks resistant embryos develop. Developing embryos are transferred to WPM supplemented with 20 g/l sucrose, 1g/l casein, 1M MES, 500 mg/l activated charcoal, 0.1 mg/l BAP, 400 mg/l carbenicillin, 150 mg/l timentin, 200 mg/l kanamycin, 0 g/ sorbitol and 8 g/l agar for germination. We are currently testing this protocol on Richter 110, 1103, 101-14 Ru 140 MGT 420A Colombard and Chardonnay using a construct containing the DsRed transgene. We have observed highly variable transformation frequencies from experiment to experiment. Some experiments result in very high numbers of resistant embryos while other experiments fail to produce any embryos. The more critical issue is that transgenic embryos that do form, although normal in appearance, have been very recalcitrant to regenerate into whole plants. Few embryos develop into seedlings while the majority fails to germinate (**Figure 2**). We will continue to test additional genotypes using this system; however, if this transformation method is to serve as a viable alternative to the transformation of stored embryos, we will need to achieve more consistent transformation frequencies of suspension cultures and more consistent regeneration of whole plants from the transgenic embryos. A summary of the experiments and the transformation frequency are given in **Table 4**. Once embryogenic suspension cultures are initiated for rootstock genotypes 3309A, and Freedom, and scion genotypes Pinot Noir, and Colombard, we will test direct transformation of suspension cultures using those genotypes.

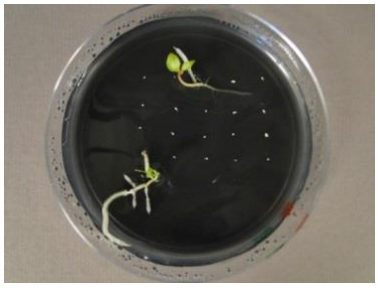


**Figure 2**. Germinating embryos from transformation of cell suspension cultures of 101-14 on WPM supplemented with 20 g/l sucrose, 1g/l casein, 1M MES, 500 mg/l activated charcoal, 0.5 mg/l BAP, 0.1 mg/l NAA 50 g/l sorbitol and 14 g/l agar and transfer to WPM supplemented with 20 g/l sucrose, 1g/l casein, 1M MES, 500 mg/l activated charcoal, 0.1 mg/l BAP, 8 g/l agar for plant regeneration. Only two of the twenty–one putatively transformed embryos on this plate, germinated after transfer to medium lacking sorbitol.

**Table 4.** Number of embryogenic colonies forming after inoculating approximately 1-2 ml of cell suspension with Agrobacterium and plating onto selection medium.

| Genotype | Number of Experiments | # Of putative transgenic embryos/ml of plated suspension | # of putative transgenic plants produced |
|---|---|---|---|
| 101-14 | 17 | 54 | 2 |
| 1103 | 20 | 30 | 2 |
| Richter 110 | 5 | 1 | 0 |
| 140 RU | 2 | In progress | In progress |
| MGT 40a | 2 | In progress | In progress |
| Colombard | 2 | In progress | In progress |
| Chardonnay | 2 | 0 | 0 |

**Objective 6. Establish in vitro shoot cultures for seven rootstock genotypes and six scion genotypes using indexed material from Foundation Plant Services (FPS) or field material from FPS and establish bulk meristem cultures for all 13 genotypes for use in transformation.**

We are maintaining disease free *in vitro* stock plants of 101-14, Chardonnay and Cabernet Sauvignon that we received as *in vitro* cultures from Foundation Plant Services (FPS). For material that was not available through FPS we have collected shoot tips from field material grown at FPS. This includes genotypes 3309C, Freedom, Richter 110, MGT 420A, 140Ru, Salt Creek 11-03, and scion genotypes Cabernet Sauvignon, Pinot Noir, Zinfandel, and Colombard. Four inch shoot tips were collected, cut into 3 inch sections, transferred to 50 ml centrifuge tubes and surface sterilized in 0.526% sodium hypochlorite for 15 minutes followed by three rinses in sterile distilled water. The shoot tip was cut into nodal sections and any tissue damaged by sterilization was removed. The nodal sections were transferred onto agar solidified Chee and Poole C2d Vitis medium containing 5mg/l chlorophenol red or agar solidified MS minimal organics medium supplemented with 1.0 mg/l BAP, 0.1 mg/l IBA, 0.1 mg/l GA3 and 5 mg/l chlorophenol red (**Figure 3**). The addition of chlorophenol red to the medium allows us to identify any contaminated shoots before the bacteria or fungus is visible based on the pH change of the medium. Aseptic shoot cultures have been established and have been plated onto Mezzetti medium with increasing levels of BAP in order to create bulk meristem cultures (**Figure 4**).

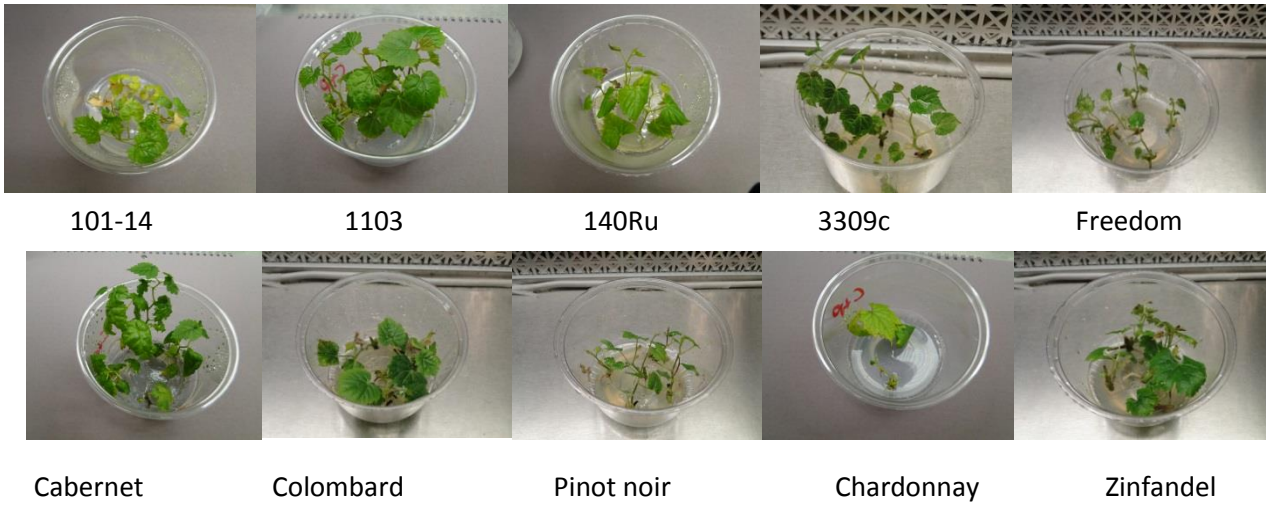



**Figure 3.** Established *in vitro* shoot cultures for use in generating bulk meristem cultures.

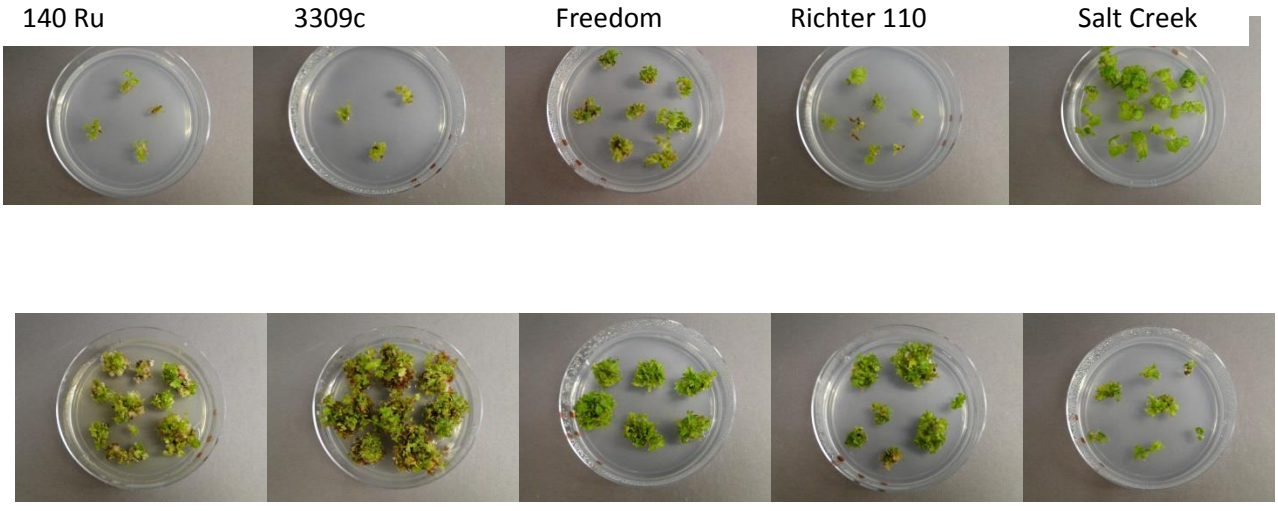



**Figure 4.** Initiation of bulk meristem cultures for rootstock and scion germplasm

**Objective 7. Test Mezzetti et al., 2002, bulk meristem transformation methodology for seven rootstock genotypes and six scion genotypes as an alternate to somatic embryo transformation.**

Bulk meristems of Thompson Seedless, Chardonnay and Cabernet Sauvignon were sliced into thin, 2mm slices and inoculated with Agrobacterium strain EHA105 and co-cultures on Mezzetti medium supplemented with 3 mg/l BAP in the dark at 23 degrees centigrade. After three days, the thin slices were transferred to Mezzetti medium supplemented with 3 mg/l BAP, 400 mg/l carbenicillin, 150 mg/l timentin and 25 mg/l kanamycin sulfate. After three weeks tissue was transferred to the same medium formulation, but the kanamycin level was increased to 50 mg/l. After an additional three weeks the tissue was transferred to medium of the same formulation but the kanamycin level was increase to 75 mg/liter. Subsequently tissue was subcultured every three weeks on medium containing 75 mg/l kanamycin. Since the construct used to transform the bulk meristems contained DsRed gene, we were able to monitor transformation efficiencies in real time. To date, we have only been successful producing transgenic shoots from bulk meristems of Thompson Seedless. Twenty four of the 75 thin slices sections of Thompson seedless produced DsRed sectors and three of these regenerated into shoots. We were able to produce DsRed expressing callus on Cabernet sauvignon and Chardonnay, but none of this tissue regenerated into shoots. In our findings, the use of kanamycin at 75mg/l appears to be suboptimal for selection. Although we did identify a limited number of DsRed shoots for Thompson Seedless, many additional shoots which developed on selection medium containing 75 mg/l kanamycin were non-transgenic based on DsRed expression. If not for the use of the scorable marker DsRed, we would not be able to distinguish the true transgenic shoots from the non-transgenic escape shoots until they were transferred to rooting medium with kanamycin. We are now repeating these transformation using higher levels of kanamycin, starting at 75mg/l and increasing to 150 mg/l. A summary of the bulk meristem transformation experiments initiated to date is given in **Table 6.**

**Table 6 Summary of bulk meristem transformation experiments**

| Experiment | Date | Genotype | Plant Selection | Explant |
|---|---|---|---|---|
| | | | | |
| 169022 | 1/22/2016 | Thompson Seedless | kanamycin | Bulk Meristems |
| 169024 | 1/22/2016 | Cabernet sauvignon | kanamycin | Bulk Meristems |
| 169025 | 1/22/2016 | Chardonnay | kanamycin | Bulk Meristems |

**Table 7** Summary table providing the progress for each objective for each of the grape rootstock and scion genotype

| Genotype | Somatic embryos from anthers | Cell suspensions | Stored somatic embryos | Shoot tip culture | Bulk meristems | Transgenic embryos | Transgenic plants | Relative Transformation efficiency * |
|---|---|---|---|---|---|---|---|---|
| Thompson seedless | + | + | + | + | + | + | + | 10 |
| 1103 | + | + | + | + | + | + | + | 3 |
| 101-14 | + | + | + | + | callus | + | + | 5 |
| 110 Richter | + | + | + | + | initiated | + | + | 7 |
| 140 Ru | + | + | + | + | initiated | - | - | ND** |
| 3309C | - | - | - | + | initiated | - | - | ND |
| MGT 420A | + | + | + | + | initiated | - | - | ND |
| Freedom | + | - | - | + | initiated | - | - | ND |
| Harmony | - | - | - | - | - | - | - | ND |
| Salt Creek | - | - | - | - | initiated | - | | ND |
| Cabernet sauvignon | + | + | + | + | + | - | - | ND |
| Chardonnay | + | + | + | + | + | + | + | ND |
| Colombard | + | + | + | + | initiated | - | - | 1 |
| Merlot | - | - | - | - | - | - | - | ND |
| Pinot noir | + | - | - | + | initiated | - | - | ND |
| Zinfandel | - | - | - | + | initiated | - | - | ND |
| | | | | | | | | ND |

* Relative transformation efficiency on a scale of 0 worst, 10 best with 10 reflecting the transformation efficiency for Thompson Seedless
**ND- not determine

**H. Publications produced and presentations made that relate to the funded project.**

Tricoli D. M. 2015. Expanding the range of grape rootstocks and scion genotypes that can be genetically modified for use in research and product development. Pierce's Disease Symposium Report pp 223-230.

**I. Research relevance statement, indicating how this research will contribute towards finding solutions to Pierce's disease in California.**

UC Davis Plant Transformation Facility has previously developed grape transformation technology for 101-14 and 1103P; two important grape rootstocks for the California grape industry for use in rootstock-mediated Pierce's disease resistance strategy. However, if rootstock-mediated resistant strategies are to be successful deployed, additional rootstock genotypes will need to be transformed in order to adequately cover the major wine growing regions in California. We therefore are testing our grape rootstock

transformation technology on seven additional rootstock genotypes including 110R, 3309C, Harmony, Freedom, 420A, 140Ru and Salt Creek. Additionally, it is not yet known if a rootstock-mediated Pierce's disease resistance strategy will prove to confer durable, commercially viable levels of resistance to the grafted scion. If rootstock-mediates resistance proves unsuccessful, direct transformation of scion clones with disease resistant transgenes may be required. Therefore as a fallback strategy, in addition to testing the range of rootstocks that we can transform, we are also testing our existing transformation technology on a select group of scions. For this proposal we are testing our transformation protocol to six scion varieties including Cabernet Sauvignon, Chardonnay, Pinot Noir, Zinfandel, Merlot and French Colombard.

**J. Layperson summary of project accomplishments.**

UC Davis Plant Transformation Facility has previously developed a method for genetically modifying 101-14 and 1103P; two important grape rootstocks for the California grape industry. This technology will allow us to introduce genes useful in combating Pierce's disease into the rootstocks of grape and allow us to test whether a modified rootstock is capable of conferring resistance to the grafted scion. This strategy is commonly referred to as rootstock-mediated resistance. If rootstock-mediated resistant strategies are to be successful deployed throughout California, additional rootstock genotypes besides 1103P and 101-14 will need to be modified in order to adequately address the rootstock requirements of the diverse wine growing regions in the State of California. We therefore are currently testing if our method for genetically modifying grape rootstocks can be used successfully on seven additional rootstock genotypes used in California wine grape production. These include; 110R, 3309C, Harmony, Freedom, 420A, 140Ru and Salt Creek. Since it is not yet known if a rootstock-mediated disease resistance strategy will prove to confer durable, commercially viable levels of resistance to the grafted scion we are also testing our method for modifying grapes on a select group of scions including; Cabernet Sauvignon, Chardonnay, Merlot, Pinot Noir, Zinfandel, and French Colombard. We have made significant progress in establishing the tissue culture materials we will need to test our transformation strategies in these additional genotypes. To date, we have demonstrated that in addition to 101-14 and 1103, Richter can be included in the list of rootstock genotypes that we can successfully transform. The results of this work will allow for the establishment of a self-sustaining grape tissue culture and transformation service for a wide range of rootstock genotypes that can be utilized by the PD/GWSS Research Community in their research efforts. It will also establish a germ bank of cell suspension cultures and a repository of somatic embryos for rootstock and scion genotypes used in California, which can be made available to the grape research community.

**K. Status of funds.**

We anticipate that all funds allocated for fiscal year FY2015-2016 will be expended.

**L. Summary and status of intellectual property associated with the project**

Methods developed under this proposal will be employed as part of a, cost-effective grape tissue culture and transformation service that can be utilized by the PD/GWSS Research Community.